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OF
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DEDICATION OF THE HENRY BARTON JACOBS ROOM[1]

The Henry Barton Jacobs Room on the Southwest corner of the Institute floor of the Welch Medical Library was formally dedicated in the Lecture Room of the Library, on January 14, 1932, at 4 P.M. This room was designed to contain the valuable collections donated by Dr. Jacobs to the Institute of the History of Medicine, numbering upwards of 5000 medical books, about 2500 medical prints, 1000 medical medals and tokens and between 300 and 400 autograph letters of physicians. These items are distributed, for the most part, under glass, in handsome mahogany wallside cases. On the west side is an oil painting of Dr. Jacobs. Bronze busts of Ambroise Paré and Claude Bernard occupy niches on the south side. The medals are deposited in glass cases opposite the two windows on the north side. From left to right these stained-glass windows commemorate Jenner, Laennec, Pasteur and Osler as follows: The Jenner half of the west window is surmounted by a shield in stained glass, with the inscription "Vaccination" at the top and a lancet below. The panes beneath show a cow and a milk-maid, in other words, the source of the vaccine matter and the first subject of vaccination. The Laennec shield is inscribed "Tuberculosis" with the cross symbol of the Tuberculosis Societies beneath. The panes below show the title page of Laennec's book on mediate auscultation and a stethoscope. The Pasteur shield bears the legend "Immunity," with chemical apparatus. Below are a sheep and laboratory animals. The Osler shield has the word "Medicine" at the top with the Æsculapian staff beneath. The panes show the Æsculapian cock. The cases flanking the portrait of Dr. Jacobs contain his unique collection of Rabelais items. The north wall is devoted to literature on tuberculosis, the east wall to Osler items, the south wall to Pasteur, Laennec and Jenner. The overflow of this magnificent collection is deposited in the alcoves of the book-stack on the third (Institute) floor.

The ceremonies began with the following introductory remarks by President Joseph S. Ames:

[1] January 14, 1932.

INTRODUCTORY REMARKS OF PRESIDENT AMES

There is no need for me to give in detail the reasons why we are gathered in this Hall this afternoon. You know that Dr. Jacobs is to present formally today to the University his wonderful collection of books, pamphlets, prints, letters and medals dealing with the History of Medicine; and that Mr. Randall, acting for the Board of Trustees of the University, is to accept formally this gift. Before

FIG. 1

these actions are taken, several friends of Dr. Jacobs and the University will tell us something in regard to the nature of the gift and the reasons for its being made to the University.

I do not remember any occasion which has given more pleasure to more people than this one in which we are taking part today. Certainly all the officers of the University and the Hospital are delighted, and this must be equally true of the friends of both institutions; but I beg you to think also of the pleasure that is Dr. Jacobs'. He has

had, first of all, the great joy of making the collection, and the delight that comes to a collector who has a definite purpose centered in a scholarly instinct is one of the greatest in the world when he succeeds in securing rare items, often after a long search. Dr. Jacobs also has a greater pleasure today than any of us, because it is truly more blessed to give than to receive; and in the future he will have the satisfaction of seeing what great use this collection is to students and scholars, not alone of this University, but from all over the world. Truly he is a happy man.

It is not my privilege to be able to do more than to express to him our keen appreciation and our sincere gratitude.

Dr. William H. Welch then followed with felicitous observations on the princely character of Dr. Jacobs' gift to the Institute and its significance for the future prosecution of medico-historical studies.

REMARKS OF PROFESSOR HARVEY CUSHING

Once upon a time, long, long ago—almost a third of a century—there were three men who lived as happily together, on what little they had, as did Taffy, the Laird, and little Billee. There was primarily *the bearded one*, then *the bushy-browed one*, and finally *the nondescript one*, who had nothing beautiful and distinguishing about him and so belonged to the common herd of men. They dwelt on a side street of a most pleasant town, in a rented, four-decker house, on whose door appeared three shingles so well-polished as to intimate to the unwary that they were doctors of long experience. But as the bushy-browed one alone had that look of wisdom, which lured patients to him even against their better judgment, to him was apportioned the ground floor—properly speaking, C deck. To the nondescript one, who was least likely ever to be consulted, A deck was given over; and the bearded one, who was in a state of expectant hopefulness, occupied B deck, with an office up only one flight.

This, was the general arrangement, though the eye-browed one, because of his nearness to the landing stage and his large waiting room, was obliged to sleep on A deck and to bathe aft on B deck. But this was not an inconvenience after he learned both to shave and to read the "Sunpaper" *en route*. There was still a lower deck, of Stygian

character, which, when once inspected with a lighted candle, was found to be much more densely populated—but this may be passed over as only remotely bearing on the fable.

One peculiarity of the abode was that the possessions of the owner, including innumerable mural cabinets and book cases which were carefully locked against prying fingers, left no wall space for the three tenants who, therefore, in their early days found it convenient to possess only one book apiece—a textbook of medicine presented to each of them by the man-next-door.

You are, perhaps, beginning to suspect the locus of this four-decker house, but you can have as yet no possible suspicion of who were its occupants. They differed considerably, not only in outward appearance and corresponding desirability as doctors, but in the way in which they did their marketing—a responsibility which was assumed in weekly rotation. The bearded one did his by telephone at a place with the jumpy name of Happer Mac Something, which was costly. During the succeeding week, the eye-browed one endeavoured to economize by sending a person named William to market, which left him no time to clean the steps and polish the shingles, which was bad for business. Hence, to even up on the third week, the nondescript one, like the little pig, went himself to market, purchased his gudgeons and chipped beef and cried "wee, wee, wee" all the way home.

Now this was all very pleasant and delightful and might have gone on forever had it not been for two things; *one* was that the expensive bearded one and the penurious nondescript one began each of them to cast an eye about for some one who might be persuaded to do their marketing for life. The *other thing* was the man-next-door.

For he surreptitiously at about this time began leaving catalogues of second-hand books on decks A, B, and C—one at a time at first and then in such numbers they could not be overlooked. The eye-browed one only had time to read them in bed, but the unemployed twain were at it—when not marketing—pretty much all day. Each two would warn the third to beware—to yield meant the beginning of the end—read the damn things but never send orders.

It was in vain. Each got the infection for which, like the common cold, there is no known preventive. So when mysterious brown paper parcels with foreign stamps began to arrive, there was, of course,

some mistake—probably ordered by the man-next-door. But finally, when something all three had secretly ordered, each having previously warned the other "not to think of such a thing—the very idea of one pound ten for such a trifle and last month's market bills unpaid!"; when, I began to say, some item under these circumstances came by post to one of the three, there would, respectively, be gnashing of teeth and pulling of beards and eye-brows.

This, alas, however mournful to relate, was the beginning of the end. The bearded one said he could endure it no longer—he would take to himself a wife who would not double-cross him in his catalogomania. It was very sad, but the nondescript one soon followed suit, and then the man-next-door, who was the real cause of the mischief, fled the country, and it is easily understood what, in turn, the eye-browed one was obliged to come to.

But the point of the legend is, that the bearded one, because of his peculiar method of marketing by telephone, got a grasshoppers' start over the other two, and that is the chief reason, my dears, why we happen to be here today, listening to this old Æsculapian fable whose moral is clear.

REMARKS OF DR. F. H. GARRISON

Mr. President, Mr. Randall, Dr. Jacobs, Dr. Welch, Ladies and Gentlemen:

This gift of Dr. Jacobs is the latest and most munificent of a considerable line of similar donations, acquired by the Welch Medical Library as bequests from the estates of heads of departments and chiefs of service in the Medical School and the Hospital. If we consider for a moment these gifts, laid at the feet of the University by her faithful servants of so many years' standing, we realize that the expression *alma mater* is no empty phrase, that it really betokens a benignant mother, not the grim, gaunt, forbidding stepmother or maiden aunt of bygone tradition, for whom Lord Berners, pupil of Stravinsky, has written burlesque funeral marches. The princely quality of Dr. Jacobs' gift, recalls the first line of the first ode of Horace—"Maecenas atavis edite regibus." It is a donation to the Institute of History of Medicine, which has existed to date as a kind of orchid or air-plant in the top of the growing tree which is the Welch

Medical Library. To all practical intents and purposes, both Institute and Library are beneficiaries of Dr. Jacobs' gifts. In a period of such financial stress and strain as this, we are not gathered in the spirit of those who would fiddle while Rome burns, but in appreciation of a lucky windfall in an unlucky period. As Billings observed at the Centennial Dinner of the College of Physicians of Philadelphia in 1887, it takes strong legs to support prosperous days, and an institution of this, or any similar kind, cannot afford to stand still for more than a week together. This Institute, too, has passed through its period of "inspissated gloom," in which accomplishment has seemed in inverse ratio to income. But in the teaching of the history of medicine by didactic lectures, considerable advance has been made through the contributions of that lovable gentleman, Sir D'Arcy Power, through the success of Dr. Oliver's lectures to the pre-medical group at Homewood, and those of Professor Sigerist. It is to Homewood, evidently, that we must look for voluntary effort in advanced research work. To date, the purchases of books for the Institute have been largely borderline material, reflecting, to some extent, the dictum of John Lothrop Motley: "Give me the luxuries of life, and I will dispense with its necessaries." But of these necessaries, in other words, of the basic and classic texts of medical literature, the reference books and indispensable monographs, there is a goodly supply on hand among the gifts and bequests of Drs. Howard A. Kelly, Henry M. Hurd, William S. Halsted, William H. Welch, William G. MacCallum, John Whitridge Williams, Rupert Norton and Henry Barton Jacobs. In consideration of the gift of Mrs. Rupert Norton, now of four years' standing, the Seminary Room of the Institute will be dedicated in the near future as the Norton Room. Very typical is the bequest from the estate of the late Dr. Whitridge Williams, a well-nigh perfect assemblage of the fundamental classics of obstetrics. In a quiet, unobtrusive way, without fuss or feathers, press agents or other advance agents of prosperity, these gentlemen have kept the sacred fires burning, and such collections stand in evidence of their sustained and sincere interest in the history of their profession. Most important of all, in magnitude and prospective value, is the collection of Dr. Jacobs, hitherto known only to his most intimate friends. Here it will stand as an index of his lifelong devotion to his profession, of his devotion to

the civic, intellectual and charitable interests of Baltimore, of his unfailing hospitality to his professional brethren, and of his feeling for the social amenities and the finer graces of life.

DEDICATORY ADDRESS OF DR. JACOBS

Mr. President and Ladies and Gentlemen:

I think I may call you all *friends* in that you have come so generously to partake of my pleasure in this occasion, and particularly I am grateful to Dr. Cushing and Dr. Viets for journeying all the way from Boston to help me in celebrating this happy day.

I hardly know what to say, but it occurs to me you may perhaps be somewhat interested in hearing of the inception and growth of the Collection. In a way it may be looked upon as an objective autobiography of my life in the past forty years, not by any means the chief interest of these years, but rather an *outgrowth* of a more important interest which has claimed much of my attention. This main interest as well as this outgrowth had their beginning in a circumstance which briefly I will relate, though it involves a personal subject that I speak of with some hesitancy.

About the year 1892 or '93 my only brother, and the only other child of my parents, suddenly had a very severe hemorrhage of the lungs. He was six years my junior and so I had feelings for him somewhat beyond those merely of a fraternal nature. He was the baby of the family, and I, as an elder brother, was much interested in guiding his life to a worthy career. He had finished Exeter, graduated with honours at Harvard, made a distinguished course in the Harvard Law School and entered one of the best known and most conservative law offices in Boston where his future seemed secure, when after some months this sudden and alarming hemorrhage occurred. Of course, it was tuberculosis. He came to Baltimore, and Dr. Osler sent him to Augusta, Georgia. Here he passed two or three successive winters, alternating in the summers at Saranac under Dr. Trudeau's care. He improved greatly for a time, but in the summer of 1896 he declined rapidly and was sent home from Saranac to die. Here began my interest in tuberculosis which you will all recognize as the subject with which my name has been most intimately associated.

Starting then with this interest in tuberculosis as a central point, the whole Collection which I have gathered together is fore-shadowed; all of its features may be said to have radiated upward and downward to the right and to the left from this centre.

Associated with Dr. Osler, and greatly under his inspiring influence, I was first led to do what I might to bring to the attention of the community the nature of this disease and the means for its prevention. In this effort I became active in the so-called Anti-Tuberculosis Crusade. It followed naturally that I must study and own books relating to the disease, and so one of my earliest purchases was that of the Frenchman, *Laennec*, who had written the first comprehensive account of the nature and extent of the disease. I secured a first edition of Laennec's book in which he not only maintains the unity of tuberculosis wherever tubercles may appear in the body, but also offers a new instrument, the stethoscope, for its earlier and more exact determination when located in the lungs. This book became the nucleus of my Collection—*Laennec* became my first *hero*. To learn the influences brought to bear upon him, I must look backward to Corvisart, to Bichat, to Auenbrugger, even to Hippocrates. To learn the results of his teaching I must go forward in France to Louis, to Andral; in this country to Morton, to Gerhard, to Bowditch, to Flint, to Welch, and in Europe to Villemin, who determined that tuberculosis was transmissible by inoculation; to Pasteur, to Koch, who happily discovered its ultimate cause, and thence on to all the studies by innumerable men in the application of the knowledge acquired. Laennec's original book was of such importance that it early was translated into various languages. John Forbes in England made the first English; soon an American edition followed, also a Belgian, German and Italian, and these had to be sought.

In 1826 Laennec issued a second edition in which he entirely transformed the order of subjects and made the book a much more complete treatise on Diseases of the Lungs rather than a mere chronicle of the signs of disease as determined by the stethoscope. This edition, this book, may well be considered the beginning of *modern medicine*; such importance does it possess in introducing a new conception of disease and new methods for its study. A prize was offered in that year, 1826, by the French Institute for the best medical book of the year. Where-

upon, Laennec determined to offer his new edition for the prize, and that he might have a friend at Court, he wrote his friend, Ampère, the student of electricity, a member of the Institute, suggesting he use his influence in securing for him, [Laennec] the prize. You will see this precious letter in one of the show cases in the lobby. Unfortunately, for the technical reason that the book was not offered at the proper date, Laennec failed to receive the prize. He was grievously disappointed and immediately afterwards left Paris for the country, where in two or three months he died of tuberculosis with which he had been struggling for several years.

The hope held out by Koch's discovery that tuberculosis might be eliminated, as had been small-pox, led me in my collecting to Jenner and his beneficent discovery of the efficacy of Vaccination of Cow-pox lymph as a preventive of small-pox, so Jenner became my *second Hero*; and again he, as a centre, demanded all the books which preceded him and all that followed. I wish I could say that *all*, both before and after, were to be found in my Collection. Of course, this is not possible, but there are many interesting items, notably complete editions of his writings, many of which are rare and difficult to obtain. Even I am able to show a little clipping of hair from the back of the Cow from which Jenner obtained his first lymph. The possession of this rather trifling but sentimental item I owe to my friend, Dr. William T. Howard, Jr., who had it from his uncle, who obtained it from Dr. Miles, who in turn had received it from the curator of the Museum of St. Thomas's Hospital in London where this cow's skin is sacredly preserved. Preceding Jenner, *inoculation* of small-pox from one individual to another had been practised with the idea of producing a light attack to obviate a severe one. This method existed from the time of Mary Worthy Montague to that of Jenner. There is a large literature on the subject, most of which Dr. Klebs has collected. He has intimated that he may give this collection to the Welch Library to supplement my Vaccination Collection. If he does, this Library will be very rich in preventive Small-pox literature. As I have already intimated, Pasteur followed in the train of Laennec, and he became my *third Hero*. There is to be found in the Collection much of his work in its original form. I might say that when an exhibition of Pasteuriana was proposed in New York to celebrate the Centenary

of his birth, I was able to send for display thirty-five separate titles which were not to be found in that city.

It goes without saying that one could not become interested in these various lines of collecting without becoming exceedingly interested in the whole subject of the History of Medicine, and so much will be found in my Collection relating to that subject, from the early historians in Europe to Clifford Allbutt and Osler in England, and from Thacher in his country to our own distinguished Garrison.

Early I was attracted to the great humanist, Francis Rabelais, and his unfortunate contemporary, Servetus; and a large section of the shelves will be found to contain many editions of Rabelais' work. The smallest book in this Collection, and one of the rarest, is the 1545 Lyons edition of Rabelais' "Aphorisms of Hippocrates." Also, will be found my first Rabelais purchase, the highly esteemed *1741* edition, which I found in Tours. There is, moreover, a complete series of the Rabelaisian studies which have been published in recent years. This runs to many volumes and indicates the great interest many scholars take in the old story-teller of Chinon.

It is but a step from interest in an author's book to become interested in that author as a person, and so my collecting has led me to seek portraits of the leaders in medicine. These portraits are to be found in photographs and engravings, in medals and tokens even in busts in bronze. The Collection is, therefore, rather rich in this respect. In the study of the characters of men, we first examine their books, and then their faces as portrayed in their pictures, so also we examine their handwriting, for in *it*, often more than in either of the other two, the true nature of the individual is made evident. Therefore, I have felt no Collection was complete which did not contain the autograph letters or manuscripts of the authors in which we were interested. Furthermore, we never get so close to a man or woman as when we are touching and reading a letter or a note which they themselves have breathed upon and penned with their own hands. We are then in the most intimate contact possible with our author or friend, if he be no longer living. Take, for instance, the letters of Edward Jenner, of which there are over one hundred in the Collection; it is only necessary to look at his carefully written pages to realize, if the beautiful portraits which exist of him did not already express the same thing, what a

noble, kindly, generous man he was; the friend of the great John Hunter, so modest and benevolent with but the single thought in mind of wiping out of the world the greatest of all human plagues—and so my Collection contains many autograph letters and manuscripts. I have already referred to the precious and touching letter of Laennec to Ampère; there is also a completely unique consultation of Laennec in a case of stricture of the œsophagus. So far as I know, no other such manuscript of Laennec is known. It was greatly desired by the Faculty of Medicine of Paris, but I preferred that it should become the property of the Welch Medical Library.

It may be interesting to you to hear about the sources of the Collection, where it has been found, and now acquired. From the first, two dear friends, so long as they lived, were greatly interested in the collection and gave to it several highly prized items. I refer to Dr. Osler and Dr. Rupert Norton. Of course, the collection has been of slow growth. It began some forty years ago and was largely acquired in my various visits to Europe. Charles Boulangé, a book dealer of Paris, was of great assistance. He became interested, and in my repeated trips to Paris, before the War, he nearly always had laid by for me books, medals and prints. It is to him that I owe the Laennec letter and Consultation and the unique protrait medal of Mme. Vallery-Radot, the daughter of Pasteur. Through him she herself gave me this portrait. He also furnished me with the large collection of French theses on tuberculosis which are probably only outnumbered by those in the Surgeon General's Library.

My medals, for the most part, came from Monsieur, Madame and Mademoiselle Boudeau of 11 Rue Rameau, Paris, a little shop on a small street opposite the Bibliothèque Nationale. I discovered this place by mere chance as I was roaming about Paris. They lived in a tiny shop, depending for a livelihood upon the sale of medals. Three more worthy or kindly people could not exist; they represented the true culture, gentleness and uprightness of the French people which unfortunately Americans so seldom see in their visits to Paris. Monsieur Boudeau has died, but his widow and daughter still carry on the business.

Many of my autograph letters of Jenner have come from Maggs Bros. of London, and other London dealers have added through per-

sonal visits and by catalogues to the Collection. Germany, Holland and Italy have furnished some important items, always by catalogues. A comparatively small part of the Collection has been found in America, though the Benjamin Waterhouse, the Benjamin Rush, the David Ramsay, and other American authors have been picked up throughout the United States, notably in Boston, Washington and Norfolk.

For the cataloguing and arranging of the Collection I am wholly under obligation to my secretary of many years, Miss Koop. She has been indefatigable in her work in keeping the Collection in order and in arranging it on the shelves.

My books number 5000 or more; my medical prints to about 2500; my portrait medical medals, tokens, etc., to about 1000, and my medical autograph letters to some 300 or 400. These with the room in which they are held with its furnishings, I am only too happy to be able to give to the Johns Hopkins University for the use of the Institute of the History of Medicine, hoping not only that they may prove useful, but that they may prove stimulating to other collectors who may follow my example. I do not mean that this Collection shall become a static or dead Collection; I want it to be kept alive, useful and growing, and so long as I live, I shall not lose my interest in it, and when I die, if the economics of the time allow any of my substance to be left, I shall hope to make a small endowment, sufficient in a very modest way, to maintain and enlarge the Collection.

To you, Mr. Randall, as representing the Trustees of the Johns Hopkins University, I hand the keys to my gift, at the same time extending to my dear friend, Dr. Welch, my sincere thanks for the privilege he has given me of placing my gatherings in his splendid Library.

ACCEPTANCE OF A GIFT BY THE UNIVERSITY, ON BEHALF OF THE BOARD OF TRUSTEES, BY MR. BLANCHARD RANDALL

Dr. Jacobs:

Listening to your presentation of the "*Jacobs Room*" in this building, jealously held by the University, along with the man whose name is inscribed above its portal, I am struck with the tender care you express in handing over these useful, these charming and these valuable objects.

Your reference to the books, portraits, medals and other possessions, show with what years of attention you now regard them! Each object in the Room, from the selection of the wood for the walls and cases, to the smallest of the medals, has had your careful study. Each book you have handled as an old friend, each portrait recalls a life-history; the stained glass in the windows tells a tale of famous makers of the History of Medicine.

Every point and every article had your thought—they were not gathered by others, to be passed through your hands *en bloc*, but each represents itself along with what you would wish it to be—the best of its kind, given with your desire for excellence.

Future generations of your professional brethren, in their study of these treasures, will doubtless add another name to those who inspired a continual advance in their history.

In the name of the Johns Hopkins University, by its Vice-President of the Board of Trustees, I accept, heartily and gratefully, this noble gift—this notable example of your unselfish devotion.

CALCIUM AND PHOSPHORUS STUDIES

IV. The Relation of Calcium and Phosphorus of the Diet to the Toxicity of Viosterol[1,2]

DAVID H. SHELLING and DOROTHY E. ASHER

From the Harriet Lane Home of the Johns Hopkins Hospital and the Department of Pediatrics, Johns Hopkins University, Baltimore, Maryland

Since the discovery by Kreitmair and Moll (1) and by Pfannensteil (2) that large doses of irradiated ergosterol are toxic to animals, numerous experiments have been recorded dealing with hypervitaminosis D. The various observers, however, are at variance as to the toxic dose and as to the substance and factors responsible for the toxicity. These discrepancies may be attributed to differences in (*a*) the potency of the viosterol, (*b*) the composition of the diets with respect to their calcium and phosphorus content, (*c*) the ages of the animals at the beginning of the experiments, and (*d*) the duration of the experiments. Thus, many of the published results are based on the use of viosterol of unknown potency and varying methods of assay; and the diets employed ranged between undescribed so-called "normal diets," through the McCollum or the Steenbock rachitogenic rations to diets consisting of "table-left-overs." The duration of the experiments, also, varies from a few days to many months.

In the study here reported an attempt was made to ascertain the relation of varying amounts of calcium and phosphorus of the diet to the toxicity of viosterol in the rat.

EXPERIMENTAL

The rats used were the offspring of healthy animals reared in our laboratories on the Steenbock-Bills (3, 4) stock diet. They were placed on the experimental diets at the time of weaning. The viosterol

[1] Aided by a grant from Mead Johnson and Company, Evansville, Indiana.

[2] A preliminary report of a part of this investigation appeared in the Proc. Soc. Exper. Biol. and Med., 28: 298, 1930.

TABLE I

Composition of diets

1. Low Ca-Optimal P

Wheat flour	40.0
Wheat gluten	5.0
Casein (purified)	10.0
Salt mixture (Ca-free)	3.5
Butter	5.0
Olive oil	4.0
Starch	32.5
	100.0

Ca = 0.012, P = 0.475 gm. per cent

2. Low Ca-High P

Diet No. 1 + 6.0 gm. per cent anhydrous Na_2HPO_4, − 6.0 gm. starch

Ca = 0.012, P = 1.780 gm. per cent

3. Stock diet (Optimal Ca and P)

Yellow corn	76.0
Crude casein	5.0
Linseed oil meal	16.0
Alfalfa meal	2.0
NaCl	0.5
$CaCO_3$	0.5

3 parts diet + 1 part powdered whole milk

Ca = 0.515, P = 0.450 gm. per cent

4. Minimal Ca-High P

(*a*) Diet No. 1 + 1 gm. per cent $CaCO_3$, + 6.0 gm. per cent anhydrous Na_2HPO_4, − 7.0 gm. per cent starch.

Ca = 0.412, P = 1.780 gm. per cent

(*b*)

Wheat flour	60.0
Gluten	1.0
Casein (purified)	10.0
Salt mixture (Ca-free)	3.5
Butter	5.0
Olive oil	4.0
$CaCO_3$	1.0
Starch	0.5
Na_2HPO_4 (anhydrous)	6.0
	100.0

Ca = 0.440, P = 1.780 gm. per cent

5. High Ca-low P (Steenbock = rachitogenic)

Yellow corn	76.0
Gluten	20.0
NaCl	1.0
$CaCO_3$	3.0
	100.0

Ca = 1.240, P = 0.250 gm. per cent

6. High Ca-Optimal P

Diet No. 1 + 3.0 gm. per cent $CaCO_3$, − 3.0 gm. per cent starch

Ca = 1.212, P = 0.475 gm. per cent

7. High Ca and High P

Stock diet + 3.0 gm. per cent $CaCO_3$, + 6.0 gm. per cent anhydrous Na_2HPO_4

Ca = 1.615, P = 1.800 gm. per cent

8. Ca-free diet

Casein (purified)	10.0
Salt mixture (Ca-free)	3.5
Olive oil	10.0
Cod liver oil	1.0
Na_2HPO_4 (anhydrous)	2.0
Viosterol (10,000 D)	2.0
Glucose (c.p.), q.s.ad	100.0

Ca = 0.0, P = 0.500 gm. per cent

9. Ca and P-free

Albumin (purified)	20.0
Salt mixture (Ca and P free)	3.5
Olive oil	10.0
Cod liver oil	1.0
Viosterol (10,000 D)	2.0
Glucose (c.p.) q.s.ad	100.0

Ca = 0.0, P = 0.0

was of known anti-rachitic potency and was incorporated in the diet in the form of a solution in oil. The dosage of viosterol was calculated in cod liver oil equivalents, i.e., the minimal amount of viosterol added to the rachitogenic diet which produced a "line test" in rachitic animals in five days equals the anti-rachitic potency of 0.25 per cent of cod liver oil in the diet.

With the exception of the rachitogenic diet, the rations employed were "complete." The essential variations were in the calcium, phosphorus and viosterol content. The compositions are given in Table I. It is seen that the diets were of the following varieties: (*a*) low calcium and optimal phosphorus; (*b*) low calcium and high phosphorus; (*c*) stock diet, optimal[3] calcium and phosphorus; (*d*) minimal[3] calcium and high phosphorus; (*e*) high calcium and low phosphorus, rachitogenic; (*f*) high calcium and optimal phosphorus; (*g*) high calcium and high phosphorus; (*h*) calcium-free and (*i*) calcium and phosphorus-free.

The dosage of viosterol, given in terms of cod liver oil potency, varied from 1 per cent of the diet to 20,000 times that amount. In terms of overdosage, the amounts of the viosterol varied from 4 to 80,000 times, since 0.25 per cent of cod liver oil in the diet is the minimal curative dose.[4]

[3] The optimal and minimal amounts for growth and maintenance.

[4] The method of assay of viosterol and cod liver oil is that of McCollum and co-workers employed by Bills (3, 4) and by us until recently. The minimal anti-rachitic curative dose of cod liver oil according to this method of assay is 0.25 per cent of the diet and is fed for only 5 days. The method differs from that of the Steenbock Foundation in that the curative diet in the latter method is fed for ten days and the minimal anti-rachitic curative dose is only about 0.1 per cent of cod liver oil. Thus, according to the Steenbock Foundation method, 1 per cent of cod liver oil in the diet constitutes 10 times vitamin D overdosage; whereas, by the McCollum method, it is only 4 times the minimal curative dose. We have found that the 10 day procedure is not so reliable as the 5 day method, because many of the animals which will gain or maintain their weight during the 5 day feeding period will lose weight in 10 days, so that healing may be due to the liberation of phosphorus from the soft tissue as the result of starvation (destruction of tissue) as well as to the anti-rachitic agent.

The viosterol dosage throughout this paper is given in terms of the 5 days method. To convert it into the Steenbock Foundation units the given dosage is multiplied by 2.5.

RESULTS

1. (*a*) *Low calcium-optimal phosphorus diet.* Animals on this diet did not grow well, but survived longer than animals getting a very high calcium and the same amount of phosphorus and viosterol in the diet. With very large doses of viosterol another factor is brought into play—that of removing calcium from the bones and shifting it to the blood. On large doses the animals became emaciated and developed high serum calcium concentrations and extreme osteoporosis. In some, the osteoporosis gave the appearance in the X-ray picture of the pseudo-rachitic metaphysis described by Gyorgy (5). On intermediate doses, fed for long periods, the animals showed only slight calcic deposits in the aorta and kidneys. Animals fed 100 per cent viosterol for 340 days showed only the slightest evidence of calcification in the aorta, while the kidneys were normal.

(*b*) *Low calcium-high phosphorus diet.* Animals fed this diet also did not grow well, as the diet without the viosterol is faulty in two respects. It is extremely low in calcium and very high in phosphorus. On the larger doses of viosterol the animals did not survive long and had slight calcification of soft tissues. On 5, 100, and 1,000 per cent viosterol, fed for 220 days, the rats showed only slight calcic deposits in the cortico-medullary zones of the kidneys. However, these renal lesions were also found in the animals receiving this diet without viosterol and apparently the lime deposits were due to the constant excretion of phosphorus by the kidneys. Similar renal lesions have been described by McKay and Oliver (6) in rats fed high phosphorus diets without an excess of vitamin D.

(*c*) *Steenbock-Bills stock diet.* The well being and duration of life of the animals on this diet were indirectly proportional to the dosage of viosterol. Rats fed this diet with 10,000 or 20,000 per cent viosterol died very quickly and showed little, if any, evidence of metastatic calcification. At autopsy the intestines were filled with a yellowish, slimy material, the subcutaneous and abdominal fat was scanty and the bladder urine frequently contained blood. On smaller doses the animals did well at first, then lost weight, declined in health and became emaciated. At autopsy they presented the typical lesions described by Kreitmar and Moll, Pfannensteil and others (7, 12, 17),

namely, metastatic calcification in the soft tissues. Those fed 500 and 1,000 per cent viosterol were killed at 10 months and all showed fairly advanced calcification of the kidneys, heart and blood vessels. Those receiving 100 per cent viosterol for the same length of time were free of metastatic calcification. The latter reproduced several times and some of their offspring were successfully reared on the same diet.

(*d*) *Minimal*[5] *calcium-high phosphorus diet.* The animals in this group were very susceptible to viosterol overdosage. They died quickly and showed most pronounced calcification of the soft tissues even on 100 per cent viosterol. With the exception of those not receiving viosterol or receiving only 5 per cent, all the animals of this series died or had to be killed at three months or sooner because of their bad condition. Those receiving no viosterol or only 5 per cent were killed at 216 and 248 days. They had extensive calcification in the cortico-medullary zone of the kidneys and peppered calcic deposits throughout the renal substance.

(*e*) *High calcium-low phosphorus diet.* (The Steenbock rachitogenic ration.) Since the usual length of life of rats on this rachitogenic ration is about 60 to 120 days, and since the animals do not grow well on this diet, the inferior growth and the deaths in this group cannot be attributed entirely to viosterol. On the contrary, the lives of some of the animals were prolonged for long periods by adding viosterol to the diet. Two animals lived on this diet plus 20 times overdosage for 258 days; and two survived on 2,000 times overdosage for 394 days without evidence of abnormal calcification of organs. In high dosages, however, the addition of viosterol was quite fatal. But very few of the animals developed metastatic calcification, although they showed hypercalcemias and degenerative changes in the soft tissues.

(*f*) *High calcium-optimal phosphorus diet.* (The amount of phosphorus is about twice that contained in the Steenbock rachitogenic diet.) Animals on this diet receiving viosterol died much sooner than those on the low calcium or the high calcium-low phosphorus diets. Those on very high viosterol dosage died too soon to show calcification of organs, but those on the smaller doses which survived longer showed marked calcic changes in 150 days.

[5] The calcium-free diet mentioned by Harris and Innes (9) and by Watchorn (10) were not calcium-free, since the corn and gluten which it contained had about 40 to 50 mgm. per cent calcium.

(*g*) *High calcium-high phosphorus diet.* When the excess phosphorus of the diet was balanced by an increase in the calcium content, viosterol was somewhat less toxic than when the calcium of the same diet was held at optimal levels. Thus 100 per cent viosterol was rapidly fatal to the rats in the groups receiving the optimal calcium-high phosphorus diet and relatively innocuous to those receiving the high calcium-high phosphorus diet. This difference in toxicity may be explainable on the grounds that the absorption and the excretion of calcium and phosphorus proceeds more normally when the ratio of the two approach 1, than when it is disproportionate. Very high doses of viosterol were quite toxic, but on doses not exceeding several hundred times the therapeutic level the rats attained fairly good growth and reproduced in a number of instances. When they were killed at 266 days, the only pathological lesions found were moderate calcification in the cortico-medullary zones of the kidneys and stones in the pelvis of the kidney. The renal lesions were also present in the animals receiving the same diet without viosterol.

(*h*) *Calcium-free-optimal phosphorus diet.* Only large doses of viosterol were used. The diets, without viosterol, were fed the animals for a few days in order to eliminate the possibility of absorption of residual fecal calcium. Then 20,000 per cent viosterol was added to the diet and fed to the animals until they showed evidence of ill health as indicated by a rapid loss of weight and loose stools (5 to 23 days). The animals were then killed and autopsied. Their blood serum was analysed for calcium and inorganic phosphorus. In spite of the complete absence of calcium in the diet, the animals had moderate hypercalcemia and moderate calcic changes in the soft tissues. The bones appeared osteoporotic.

(*i*) *Calcium and phosphorus-free diet.* The results with this diet were similar to the above except that the serum calcium levels were somewhat higher and the incidence of abnormal calcification was greater. The explanation for the slight differences in the effects of viosterol in the presence or absence of phosphorus in the diet is discussed elsewhere (8).

2. *Effect on serum calcium and inorganic phosphorus.* The number of chemical determinations are necessarily rather small in relation to the large number of animals used in this study (500) since many of the animals on the larger doses of viosterol died before they could be

TABLE II

Effect of diet and varying doses of viosterol on serum calcium and inorganic phosphorus

Figures in mgm. per cent

Diet	Viosterol dosage in per cent																	
	0		1		5		100		500		1,000		2,500		5,000		10,000		20,000	
	Ca	P	Ca	P	Ca	P	Ca	P	Ca	P	Ca	P	Ca	P	Ca	P	Ca	P	Ca	P
Low Ca-Optimal P	6.7	8.1			9.5	6.0	6.7	8.1			11.0	9.7			12.1	6.7	17.6		15.4	11.0
	10.8	7.8			9.0	5.3													11.8	5.0
	8.9	6.6			13.1	6.7													17.8	
	11.2	7.8																	21.6	
																			15.4	
Low Ca-High P	7.5	14.6			7.8	10.8	6.6	10.9			7.5	11.4			16.2	9.7	16.2	9.4		
Stock diet	10.0	3.5	12.0	4.5	12.2	5.0	11.8	8.0	13.7	6.1	13.0	8.5	15.0	7.1	16.4	9.8	18.4		17.2	11.2
	11.0	3.2	11.3	4.8	11.4	4.7	12.2	5.9	10.1	6.0	9.0	5.4	16.0	7.4			12.0	7.4	12.8	6.8
							11.4	8.9			9.1									
High P Minimal Ca	7.0	16.0			7.3	10.1	12.2	15.4			9.0	18.5			3.9	13.0	4.5	14.2		
High Ca-Low P	12.0	2.5	12.0	5.0	9.9	5.6	13.0	7.0	14.0	4.0			15.4	5.0	14.0	4.5	17.5	4.5		
	11.4	2.8	11.0	4.3	15.1															
	12.5	2.0	10.8	5.2																
High Ca-Optimal P							12.2	6.9			17.3	13.0			14.8	4.3	14.5			
High Ca-High P	11.4	6.6	11.5	6.8	11.9	7.6	11.8				13.6	5.2							14.1	6.9

	Viosterol, 20,000 per cent					Viosterol, 20,000 per cent			
	Ca	P	Ca	P		Ca	P	Ca	P
Ca-Free	14.6	8.5	15.6		Ca and P free	15.5	9.3	19.5	
	15.0	8.8	13.8	9.9		13.8	7.5	19.0	8.9
	9.5		10.7	9.5		12.9	8.9	15.1	8.1
	12.4		10.7	9.5		13.3	8.5	15.5	8.3
	11.0		13.6	8.5		14.9	9.8	13.8	7.9
						14.5	8.0	19.7	
						12.9		13.5	15.0
						18.9		16.7	
						19.2		15.1	9.0

bled, and of those which were bled many were in such a state of emaciation that the blood of two or more animals had to be pooled for analysis. The concentrations of the serum calcium and inorganic phosphorus of the different groups are shown in Table II. It is seen that with the larger doses of viosterol there is a definite tendency for high values for both calcium and phosphorus. In the case of diets which tend to increase the serum inorganic phosphorus and decrease the calcium, the addition of large amounts of viosterol definitely increased the serum calcium to normal or even to hypercalcemic levels, a result in accord with those of Hess, Weinstock and Rivkin (11) and of Shohl, Goldblatt and Brown (12). In two instances, in spite of viosterol overdosage, the serum calcium levels were very low, but the animals did not exhibit symptoms of tetany. These, however, were fed the high phosphorus diet with 5,000 and 10,000 per cent viosterol and they had most extensive calcification of the aortae and kidneys. A similar hypocalcemia without tetany and marked metastatic calcification was observed in parathyroidectomized rats (13).

Hypercalcemia seems to be more frequent in animals on large doses of viosterol before extensive abnormal calcification has set in than in those in which abnormal calcification is already quite marked. Evidently, the higher serum calcium levels were encountered in rapidly fatal cases of viosterol overdosage,—before the excess calcium and phosphorus in the blood could be precipitated out in the soft tissues; and the lower serum calcium levels were seen in animals kept on smaller doses of viosterol for long periods, in which there was time for the excess blood calcium and phosphorus to deposit in the soft tissues.

A few of the hypercalcemic sera were ultrafiltered and the percentages of ultrafiltrable calcium were found to be within the normal values,—42 per cent. The serum proteins, however, were not determined and, hence, it is impossible to state which portion of the serum calcium was increased.

3. *Effect on growth and weight.* The growth and weight curves of the animals in the different groups are shown in Figures 1, 2, 3, 4, and 5. It is seen that with very large doses of viosterol the composition of the diet had very little influence on the weight and longevity of the animals. Most declined in weight very rapidly and died in a state of emaciation. With smaller doses of viosterol the response in growth

was influenced, to a large extent, by the composition of the diet. Thus, animals fed the stock diet with several hundred times overdosage of viosterol attained nearly optimal growth; while those fed the optimal calcium-high phosphorus diet declined in weight even on smaller doses of viosterol. With the low calcium diets optimal growths are not to be expected. Many of the animals on these diets would have died sooner, had not viosterol been included. In the case of the rachitogenic diet small or moderate amounts of viosterol had a definite

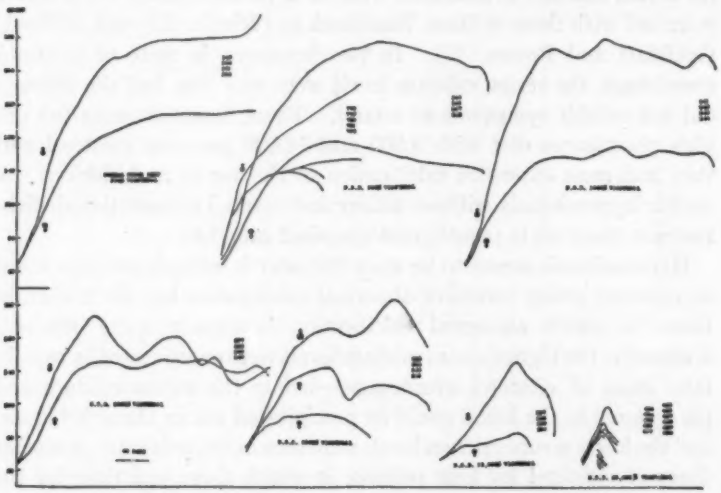


FIG. 1. COMPOSITE GROWTH CURVES OF RATS FED THE STOCK DIET AND VARIOUS DOSES OF VIOSTEROL EXPRESSED IN PERCENTAGE OF DIET EQUIVALENT TO COD LIVER OIL POTENCY

beneficial effect, since the viosterol additions increased the longevity of these animals.

The importance of long term experiments is demonstrated by the growth curves. It is seen that in many instances the animals continued to gain weight for long periods of time and then gradually began to lose weight and decline in health. If the rats had been killed on the up-grade of their growth curves, evidence of toxicity would probably have been absent. Only the prolongation of the feeding period

showed the final effects of the diets and of viosterol. Thus, the results of these experiments and those of Bills (4) cannot be evaluated in terms of short-period experiments of other investigators (9, 12, 20).

4. *Effect on reproduction.* Pregnancy and reproduction did not occur among the animals fed the rachitogenic diet. In the other groups, reproduction was irregular on the small and moderate doses of viosterol and very infrequent or entirely absent among those fed

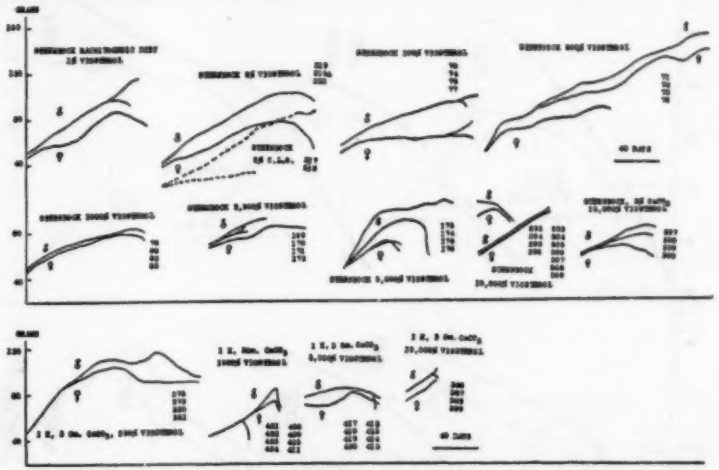


FIG. 2. GROWTH CURVES OF RATS FED VARIOUS DOSES OF VIOSTEROL AND EITHER THE STEENBOCK HIGH CALCIUM-LOW PHOSPHORUS DIET OR A HIGH CALCIUM-OPTIMAL PHOSPHORUS DIET

The latter diet contains about twice the phosphorus content of the former

very large doses, since the hypervitaminosis was rapidly fatal. On the stock diet and on the high calcium-high phosphorus diet reproduction was regular if the viosterol doses did not exceed 100 per cent, but was less regular if the doses were large. On the low calcium diets with small or moderate doses of viosterol the numbers of litters were few, the offspring were small and the incidence of "killers" (females killing their offspring) was quite marked. On the stock diet with 100 per cent viosterol two generations of rats were reared, but the spans of

life of the second generations were shorter and their growth inferior to that of their parents.

5. *Effect on offspring.* Some of the offspring of the animals fed the stock diet with varying levels of viosterol were continued on the same diets. Three offspring from the group receiving 10,000 per cent viosterol were killed in a state of emaciation at 103 days. They

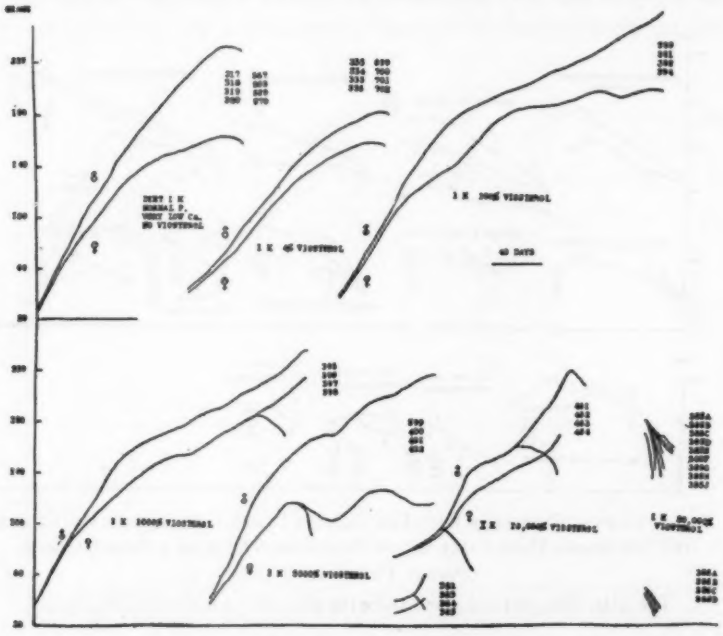


FIG. 3. GROWTH CURVES OF RATS FED LOW CALCIUM-OPTIMAL PHOSPHORUS DIETS AND VARYING DOSES OF VIOSTEROL

showed most extensive metastatic calcification and a serum calcium concentration of 18.4 mgm. per cent. Three offspring from the 2500 per cent group were killed at 186 days. The abnormal calcification of their soft tissues and the hypercalcification of their bones were more marked than in their parents who survived much longer. Greater susceptibility to viosterol overdosage in the offspring than in the

parents was also encountered in the 1000, 500 and 100 per cent viosterol groups. Of the first generation of the group receiving 100 per cent viosterol those killed at 67, 132, and 289 days showed no abnormalities, but two which were killed at 363 days had most extensive calcification of the myocardium, kidneys and blood vessels.

Further proof that the animals were affected *in utero* by large or moderate doses of viosterol was obtained from a comparison of the inorganic composition of new-born rats of parents fed the stock diet

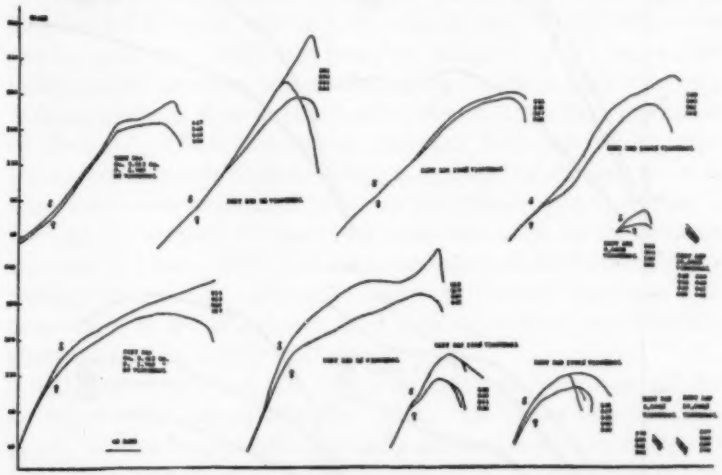


FIG. 4. GROWTH CURVES OF RATS FED VARYING DOSES OF VIOSTEROL AND EITHER THE LOW CALCIUM-HIGH PHOSPHORUS DIET NO. 33, OR THE SAME DIET TO WHICH 1.0 GM. PER CENT $CaCO_3$ WAS ADDED (DIET NO. 32)

with and without viosterol. A few new-born rats of parents fed the stock diet with 100 per cent viosterol were killed immediately after birth and the calcium of the whole animals determined by Dr. W. S. Swanson. The calcium content of these rats averaged 17.0 mgm., and those from viosterol-free parents averaged only 10 mgm. per rat,—an increase of 70 per cent,—although their birth weights were about the same.

Selye (14) observed premature calcification, fragility and fractures in

the bones of young offspring of mothers fed large doses of viosterol during the last week of gestation and throughout the lactation period. Bills and Wirick (4) have shown that the young of mothers fed large

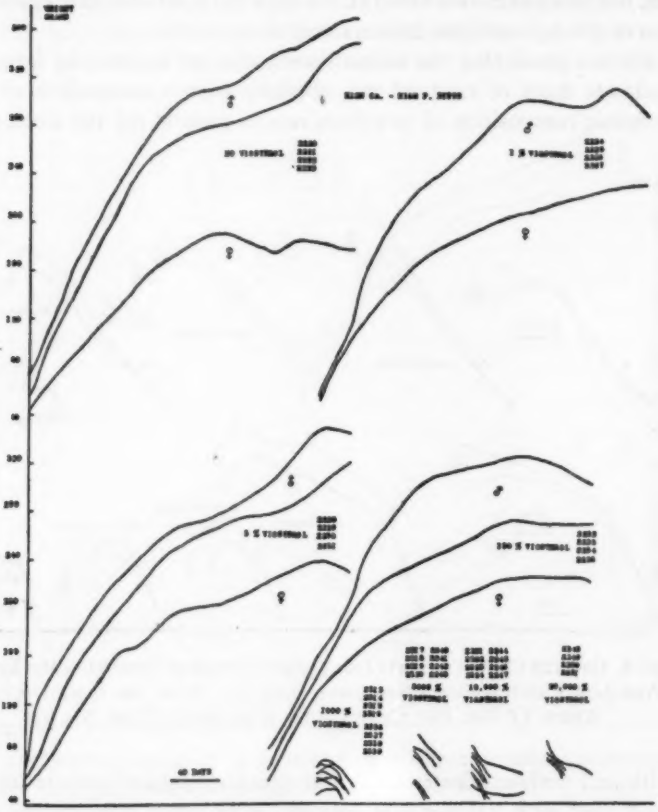


FIG. 5. GROWTH CURVES OF RATS FED HIGH CALCIUM-HIGH PHOSPHORUS DIETS AND VARIOUS DOSES OF VIOSTEROL

doses of viosterol, but suckled by foster-mothers not receiving viosterol, were as susceptible to rickets as those born of mothers fed the stock diet without viosterol. When, however, the young were suckled

by the viosterol-fed mothers their susceptibility to rickets was definitely diminished. These results would seem to indicate that viosterol, even in large doses, is transmitted through the lacteals but not through the placenta and the viosterol effects occurred only during the lactation period, although there is the theoretical possibility that the calcium, accumulated *in utero*, was expended during the period of lactation on viosterol-free milk. The chemical analysis of the newborn animals, the greater susceptibility of the offspring to viosterol overdosage and the greater resistance to rickets of rats suckled by viosterol-fed mothers show that the animals may be affected *in utero* and by lactation. While it is true that vitamin D is not transmitted in appreciable amounts through the placenta or the lacteals when only minimal amounts are fed, it is equally evident from the experiments of Hess (15), Chick and Roscoe (16), and Bills and Wirick, that therapeutic amounts of this vitamin is transferable through the milk when large doses are administered. If the transmission of vitamin D through the lacteals is dependent upon the levels of the vitamin ingested, it is conceivable that the same may hold for its transference through the placenta, i.e., small amounts of viosterol may have little or no effect on the offspring *in utero* while large doses may definitely affect the calcium content of the fetus.

6. *Effect on soft tissues.*[6] Autopsies were performed on all the animals whether they died or were killed. The tissues were examined grossly and microscopically, especially for the presence or absence of metastatic calcification. The relation of the calcium and phosphorus of the diet and the dosage of viosterol to the degree of abnormal calcification is illustrated in Figures 6, 7, and 8. It is seen that on diets low in calcium and, also, on diets high in calcium but low in phosphorus metastatic calcification was absent and, when present, it was not very extensive. When large doses of viosterol were used necrotic changes were frequently found in the liver, kidneys and heart muscle, although calcic changes were few or entirely absent. That metastatic calcification is not the sole toxic effect of viosterol was shown by Shohl, Goldblatt and Brown (12) and by Harris and Innes (9).

[6] We are indebted to Drs. W. D. Forbus and G. B. Wislocki, formerly of the departments of Pathology and Anatomy, respectively, for the preparation and interpretation of the microscopic sections of the soft tissues.

The most extensive calcification was found in the soft tissues of the animals fed the high phosphorus-optimal calcium diet. The dose of viosterol which was quite toxic to the animals in this group was

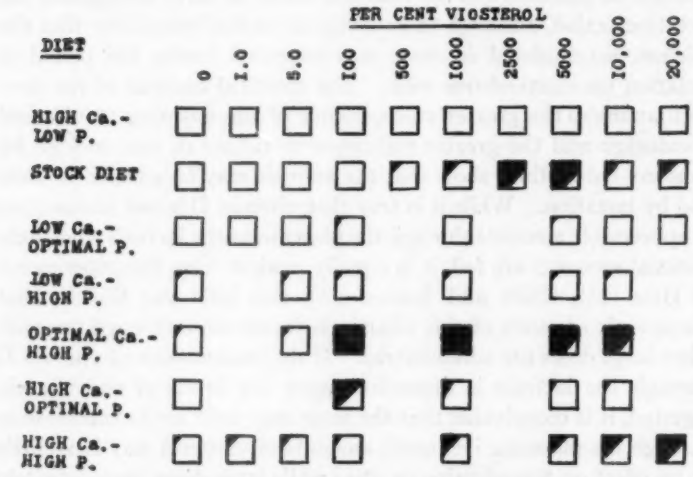


FIG. 6. APPROXIMATE DEGREE OF METASTATIC CALCIFICATION IN RELATION TO CALCIUM, PHOSPHORUS AND VIOSTEROL CONTENT OF DIETS

Blank squares indicate absence of calcification and the extent of shading in black indicates, roughly, the degree of calcification. It is seen that the optimal calcium-high phosphorus diet is most conducive to metastatic calcification, while the low calcium-optimal phosphorus and the rachitogenic diets are least conducive. The extent of metastatic calcification shown in this figure should not be confused with toxicity of viosterol, since many of the animals fed diets not predisposing to metastatic calcification showed other manifestations of toxicity such as wasting, tissue necrosis and inflammation. Some of the animals fed very large doses of viosterol died too soon to show abnormal calcification while those surviving on smaller amounts of viosterol developed abnormal calcic deposits in their soft tissues. The relation of diet and of viosterol to the degree of metastatic calcification and feeding or survival period is shown in Figure 7.

apparently innocuous to the rats receiving other diets for long periods. The calcification was marked and widespread. The kidneys were large, soft, pale and peppered with calcium deposits (Fig. 8, *B*). The myocardium contained calcic plaques, areas of necrosis, infiltration

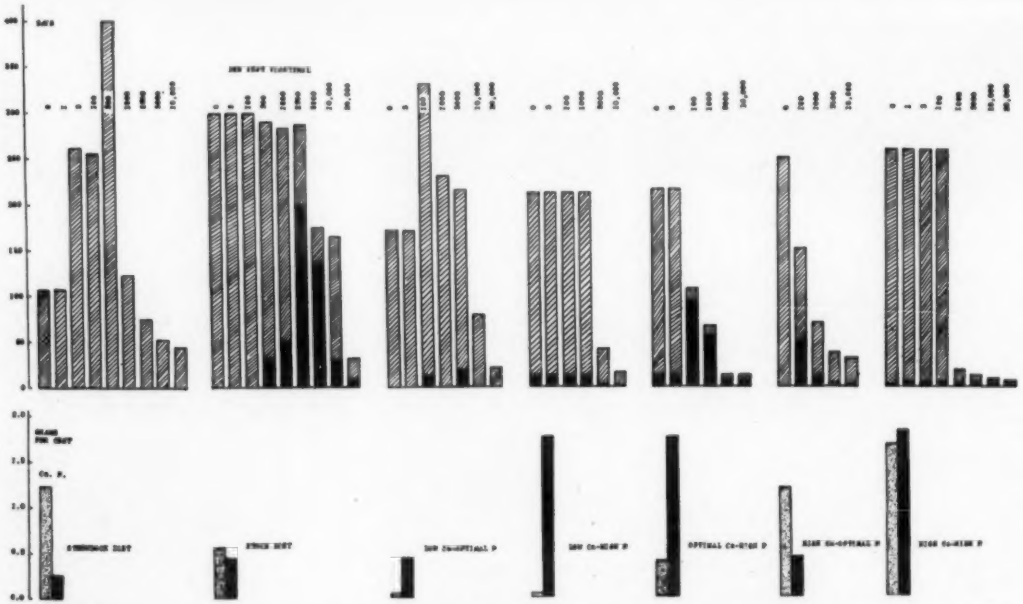


FIG. 7. RELATION OF CALCIUM AND PHOSPHORUS CONTENT OF DIET AND INCREASING DOSES OF VIOSTEROL ON THE DEGREE OF METASTATIC CALCIFICATION WHEN FEEDING PERIODS ARE CONSIDERED

The height of the blocks represents length of feeding periods and the superimposed black areas indicate the extent of metastatic calcification as contrasted to the lighter areas representing absence of calcification. It is seen that on the Steenbock high calcium-low phosphorus diet the animals survived much longer than they would have were viosterol not included in the diet; and that none showed metastatic calcification. On the other hand, the optimal calcium-high phosphorus diet produces most extensive calcification in a very short time with only 100 per cent viosterol or about 400 times overdosage. Animals kept on the stock diet and the same dose of viosterol were free of metastatic calcification even after 300 days.

and fibrosis. Calcification also occurred in the mucosa and in the muscularis of the stomach.

On the stock diet very large doses of viosterol produced toxic symptoms and death before extensive metastatic calcification took

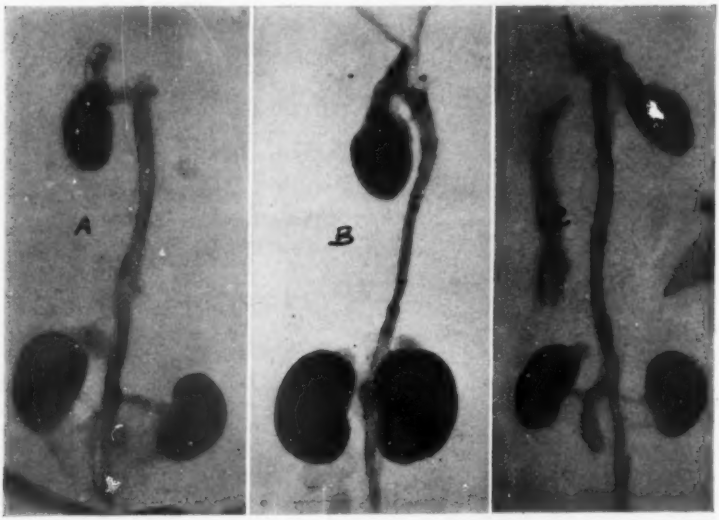


FIG. 8. THE EXTENT OF METASTATIC CALCIFICATION ON VARIOUS DIETS AND OF VIOSTEROL

The extent and distribution of calcification in the animal kept on the optimal calcium-high phosphorus diet and 1000 per cent viosterol (*B*) is more marked than in the rat kept on the stock diet and the same dose of viosterol (*A*). It is also more extensive than in (*C*) the animal kept on the stock diet with 10 times the amount of viosterol, (10,000 per cent), fed to (*A*). The contrast becomes even more striking when the time factor is taken into consideration. Thus, (*A*) was kept on the diet for 285 days, (*C*) for 160 days and (*B*) for only 60 days. Note the size and the extensive calcification in the kidneys of (*B*).

place; but on 1000, 2500 and 5000 per cent viosterol, the animals, when killed after 10 months feeding, exhibited fairly marked calcic deposits. Those fed 100 per cent or 400 times the minimal therapeutic dose of viosterol, for nearly one-third of their spans of life, showed no abnormal

calcification in the soft tissues. Some of their offspring, however, had moderate metastatic calcification when fed the diet with the same amount of viosterol for 363 days. Apparently these animals were affected by viosterol *in utero* and were more susceptible to hypervitaminosis than their parents.

In contrast to the animals in the high calcium-low phosphorus groups, those fed a similar amount of calcium but about twice the amount of phosphorus were more susceptible to the viosterol overdosage. The more extensive abnormal calcification in the animals receiving 100 per cent than in those receiving larger doses of viosterol (Figs. 6 and 7) may be due to the longer survival period of the former.

When the diet was high in both calcium and phosphorus the toxicity of the viosterol was less marked than when it was high in phosphorus by optimal in calcium. Thus 100 per cent viosterol was quite fatal to the animals fed the latter diet but harmless to those fed the former diet, in which the excess phosphorus was balanced by calcium. The only pathologic changes observed were those in the cortico-medullary zones of the kidneys, but these areas of calcification were also found in the kidneys of animals receiving this diet without viosterol.

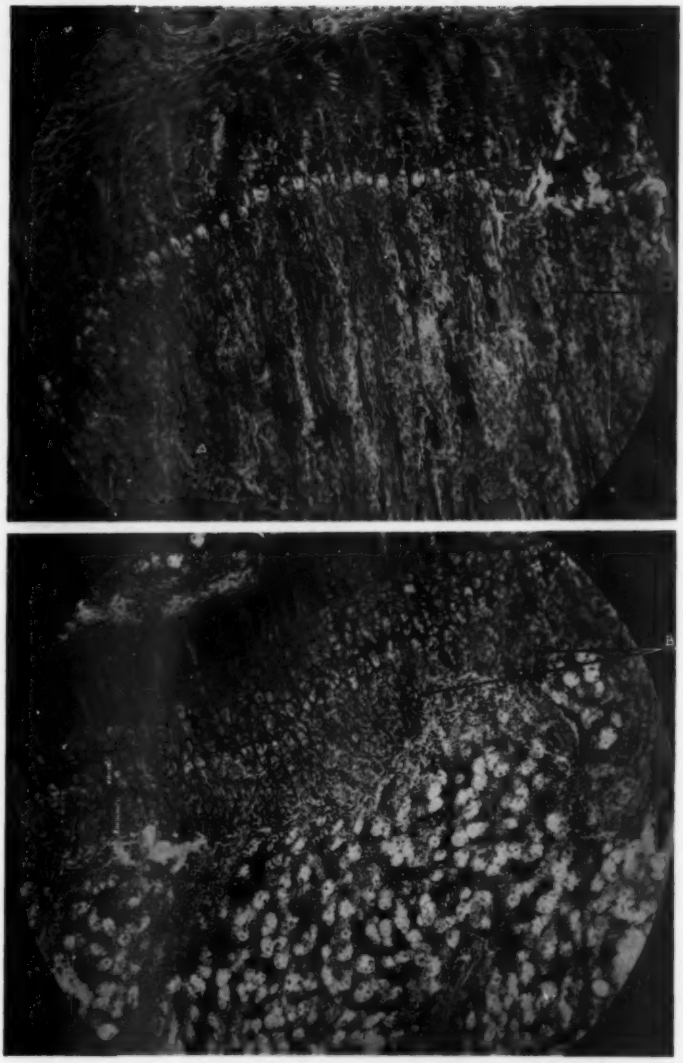
In the experiments with calcium-free and with calcium and phosphorus-free diets, 20,000 per cent viosterol was used, In spite of the complete absence of calcium or of both calcium and phosphorus from the diets moderate metastatic calcification was encountered, especially in the latter group. It is possible that, if smaller doses had been used, abnormal calcification would have been absent.

The extent of the gross and microscopic calcification in the soft tissues varied with the diet, the amount of viosterol and the duration of the experiment. In some instances the calcification consisted only of small scattered calcic plaques in the medial coats of the blood vessels or of calcium deposits in the corticomedullary zones of the kidneys, while in other cases the blood vessels were damaged nearly in their entire length and thickness and the kidneys were literally made up of lime (Fig. 8, *B*). Histologically, the vascular calcification which resembled arteriosclerosis was confined mainly to the media as in the Mönckenberg type of sclerois, rather than to the intima as in human arteriosclerosis. This is in agreement with Herzenberg (17) and others (18) who considered the viosterol

arterial calcification as a part of the general "Nekrose-Verkalkungs-vorgänge," not specific to arteries and quite distinct from arteriosclerosis. It is unfortunate that this specific type of arterial calcification due to viosterol overdosage is so frequently termed "arteriosclerosis" (19) as it conveys an erroneous impression that viosterol might be an etiologic agent in human arteriosclerosis. Not only do the lesions in the two conditions differ histologically, but the processes are entirely dissimilar and dependent upon divergent chemico-pathological disturbances.

7. *Effect on bones.* The effect of viosterol on the osseous system also varied with the diet, the dose of viosterol and the duration of the experiment. The bones of the animals fed the stock diet with small doses of viosterol appeared normal; on intermediate doses they were hard and hypercalcified; and on large doses they showed evidence of resorption. In contrast to the decalcifying effects of large doses of viosterol on the bones of the animals receiving either the stock or low calcium diets, the bones of the animals fed diets high in phosphorus and either optimal or high in calcium were hypercalcified. Apparently when enough calcium was present in the diet an excess of phosphorus predisposed to hypercalcification of the bones as well as to abnormal deposition of lime salts in the soft tissues. Similarly, the diet containing the same amount of calcium but twice the amount of phosphorus as that contained in the rachitogenic diet produced harder bones than the stock diet or the rachitogenic diets. The bones of the offspring of parents fed the stock diet and varying large doses of viosterol were unusually hard and marble-like. The shafts were thick and stubby, the trabeculae were compact and confluent and extended for some distance into the marrow cavity.

The osteoporosis which occurs on calcium-free or low calcium-optimal phosphorus diets was not improved by the addition of small or moderate amounts of viosterol. With large doses it was actually intensified. The bones were thin and fragile and showed many fractures. Roentgenologically and histologically many of these had areas of decalcification just below the epiphyseal cartilage and appeared as pseudo-metaphyses similar to those described by Gyorgy (5). When an excess of phosphorus was added to the calcium-deficient diets the osteoporosis was more marked and it was further intensified by the



FIGS. 9 AND 10. THE EFFECT OF LOW CALCIUM-HIGH PHOSPHORUS DIET AND 10,000 PER CENT VIOSTEROL ON OSSEOUS TISSUE

Figure 9 (top), is a section of a tibia of a rat fed this ration for only three days. It is seen that the trabaculae (*a*) are quite numerous, compact and extended for some distance below the zone of proliferative cartilage. Figure 10 (below) shows the section of a tibia of a rat fed the diet for twenty-six days. It is seen that there is dissolution of the matrix and nearly the entire disappearance of the osseous trabeculae. Only small fragments of calcification (*b*) are seen below the cartilaginous zone, and the entire area has been replaced by some osteoblasts and marrow cells.

addition of large doses of viosterol. Results of metabolism experiments with calcium-free diets indicated that viosterol caused an enormous excretion of calcium and a demineralization of the bones (8).

The low calcium-high phosphorus diet and 10,000 per cent viosterol were fed to a series of young rats. Two animals from the group were killed daily, beginning with the third and ending with the twenty-sixth day. Microscopic studies of the bones revealed that the osseous trabeculae, which were numerous and compact at the beginning of the experiment, decreased in number with the prolongation of the feeding period. At the expiration of the experiment nearly all the trabeculae disappeared and were replaced by remnants of osteoid, osteoblasts, and only tiny fragments of calcified material. The osseous decalcification which resulted from the diet and viosterol is shown in Figures 9 and 10.

DISCUSSION

From the experimental data presented it is evident that the composition of the diets with respect to their calcium and phosphorus content is a determining factor in the toxicity of viosterol, especially as to its effects in the production of abnormal calcification. When comparing the injurious effects of equal doses of viosterol on the animals in the different dietary groups, it appears that abnormal calcification does not occur with regularity unless there be an abundant supply of lime salts in the food. The diets most conducive to metastatic calcification were those of a high phosphorus-optimal calcium content; and the diets least predisposing were those poor in phosphorus or very low in calcium. When the diets were optimal in both calcium and phosphorus, the toxic effects were evident only when large doses of viosterol were used, or when the feeding periods were of long duration. When an excess calcium or phosphorus was added to the optimal diets the toxicity of viosterol was increased but more so on the phosphorus than on the calcium-rich diet.

The protective influence of a low phosphorus diet against viosterol hypercalcification and the enhancement of calcification by an excess dietary phosphorus indicates the importance of phosphorus in the process of lime salt deposition. The term "calcification" has gained such universal usage that in the study of calcium metabolism the

calcium is unduly stressed and the control of *phosphorus* intake is frequently neglected. This leads to confusion and contradiction in experimental results and to a misunderstanding of the physico-chemical processes involved in calcium deposition. Shohl (21) has frequently called attention to this neglect and at a recent symposium on calcium and phosphorus metabolism he (22) suggested that the word "calcification" be surplanted by a more appropriate term, embracing also phosphorus.

The importance of dietary phosphorus as a factor in calcification has been recognized in clinical and experimental rickets. Its predisposing influence as demonstrated in this study also has some clinical bearing. Following this observation and also that of Karelitz and Shohl (23) on the effect of phosphate on the healing of rickets in rats, a few children with very severe rickets were fed viosterol and inorganic phosphate for a short time. It was observed that the healing was definitely hastened by the phosphate additions.[7] One of these children had florid rickets, and epiphysitis and osteomyelitis of the upper part of the humerus. The addition of phosphate to his diet hastened the healing, not only of the rachitic, but also of the osteomyelitic process.

The predisposing effect of an excess phosphorus to calcification is also interesting in another connection. If the deposition of lime salts is due to an increase of Ca^{++} and $PO_4^{=}$ in the blood in excess of their solubility, then any disturbance which causes a continuous phosphorus retention will predispose the organism to abnormal calcification, although the serum calcium may have become secondarily diminished. Thus, in renal rickets the kidneys are unable to eliminate phosphorus and it is retained in the blood. The retained phosphorus thus tends to be excreted by the bowel as the insoluble calcium salts and it draws first upon the ingested calcium and later on that from the bones. The

[7] Viosterol was always given for a few days before the phosphate therapy was begun, since the administration of phosphate without an antirachitic agent might induce tetany in a rachitic organism as shown experimentally by Karelitz and Shohl (23). When the serum calcium is stabilized by viosterol administration, moderate doses of sodium phosphate may be given without the danger of inducing tetany. The dose of phosphate used was 3.0 gm. of anhydrous Na_2PHO_4 per day, in 6 divided doses. The phosphate is usually administered for about 10 days and then withheld for an equal period, when it may be repeated.

phosphorus retained as a result of catabolic processes in the body and also that from the food and bones sets up a vicious circle and a continuous abstraction of lime salts from the bones which result in renal rickets or osteoporosis (Mitchel (24)). The constant circulation of an excess phosphorus may also result in deposition of lime salts in the soft tissues, especially in the arteries. As such patients already have a tendency to arterial calcification the administration of anti-rachitic agents, especially on a high or moderate phosphorus intake, may intensify this tendency and, therefore, they should be used cautiously. Karelitz (25) observed that when irradiated ergosterol was given to a patient with renal rickets the rachitic process improved but the arteries showed calcification some months after the anti-rachitic therapy was discontinued. That the arterial calcification was not due to viosterol poisoning *per se* can be inferred from the fact that such pathologic changes occur not infrequently in patients with renal disease who never received viosterol or cod liver oil (26).

From the results of studies on the effect of dietary calcium and phosphorus on the tetany and serum calcium and inorganic phosphorus of parathyroidectomized rats (27) and, also, from the results of the present experiments, it would seem that a diet high in calcium and relatively low in phosphorus is best suited for patients with renal rickets. The limitation of dietary phosphorus and the ingestion of an excess calcium will tend to diminish the phosphorus retention and aid in its elimination through the bowel as the insoluble calcium salt.

The same mechanism of abnormal calcification probably operates in patients with chronic parathyroid tetany and constant high serum inorganic phosphorus levels. Although such patients are benefited by the judicious use of viosterol it is important to keep their dietary calcium high and their phosphorus intake at a minimum, since the effects of parathyroid deprivation are the retention of phosphorus and a decrease or an absence of this element from the urine (28, 29).

Another factor in the predisposition of animals to the injurious effects of viosterol and diet is the duration of the feeding period. Thus, with a given diet and with a given dose of viosterol, toxic effects may be entirely absent when the period of observation is relatively short, but may become manifest when the experiment is prolonged. Also, when comparing the effects of equal doses of viosterol under varying conditions of diet, the time element must be taken into con-

sideration. Figure 6, for example, shows that the incidence and extent of metastatic calcification is about the same in the groups receiving either the stock ration or the optimal calcium-high phosphorus diet with the addition of 500 per cent viosterol. However, when the two groups are compared on the basis of survival (Figs. 1, 4 and 9) it is seen that the latter survived only about 20 days while the former were still alive at more than 200 days.

That the presence or absence of metastatic calcification is not the only criterion for viosterol toxicity is also evident from a comparison of Figures 6 and 7 with the autopsy findings. Figure 6 shows that metastatic calcification was, relatively speaking, absent in the rats fed the low calcium-optimal phosphorus diet plus 10,000 per cent viosterol and that it was present in those fed the stock diet and an equal amount of viosterol. The autopsy findings, however, indicated that in the former group the injurious effects of the large dose of viosterol manifested themselves, not by metastatic calcification, but rather by wasting, tissue necrosis and fibrosis.

With respect to the toxicity of viosterol and the possible bearing on its clinical use in the prevention and cure of rickets, it is seen that viosterol is less toxic to the rachitic organism and that in animals reared on the stock diet the therapeutic dose is quite remote from the toxic level. Thus, animals fed this diet and 400 to 1000 times the therapeutic dose of viosterol for one-third to one-half of their spans of life, grew and reproduced normally and showed no evidence of abnormal calcification. Compared to the rat, a human being would have to be fed several thousand times the usual dose of viosterol for at least 20 to 40 years before this abnormal type of calcification would take place. Obviously, the reported toxic manifestations observed in children have been due to the use of large doses of irradiated ergosterol of unknown, and probably very high, anti-rachitic potency.

The physico-chemical considerations of the effects of varying doses of viosterol and of diet on hypercalcemia, ossification, and metastatic calcification are discussed elsewhere (8).

SUMMARY AND CONCLUSIONS

(1) The calcium and phosphorus content of the diet is a determining factor in viosterol toxicity in rats.

(a) Optimal calcium-high phosphorus diets are the most predispos-

ing to the injurious effects of viosterol; and high calcium-low phosphorus and very low calcium-optimal phosphorus diets are the least effective.

(b) On the stock diet in which the calcium and phosphorus content is optimal, dosages of viosterol several hundred to a thousand times the therapeutic dose produce no detectable injurious effects when fed for long periods. On this diet, the therapeutic dose is quite remote from the toxic level.

(2) Metastatic calcification is not the sole injurious effect of viosterol, for necrotic, degenerative and inflammatory changes without calcification occur in the soft tissues when large doses of viosterol are used.

(3) Small or moderate doses of viosterol cause deposition of calcium and phosphorus in the bones, while large doses may cause resorption of lime salts from the bones.

(4) The arterial calcification produced by viosterol is of a specific type and is limited chiefly to the media as in Mönckenberg's sclerosis, rather than to the intima as in arteriosclerosis.

(5) Viosterol is capable of raising the serum calcium to hypercalcemic levels when calcium is entirely absent from the diet and to elevate it from hypocalcemic levels produced by high phosphorus feeding.

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CALCIUM AND PHOSPHORUS STUDIES[1]

V. The Comparative Effectiveness of Viosterol and Cod Liver Oil in Protecting the Body against a Calcium Deficiency in the Diet

DAVID H. SHELLING and DOROTHY E. ASHER

From the Harriet Lane Home of the Johns Hopkins Hospital and the Department of Pediatrics, Johns Hopkins University, Baltimore, Maryland

When viosterol was introduced as an antirachitic agent, it was believed to have the same potency as cod liver oil in protecting the body against deficiencies in calcium and phosphorus. Some (1), however, have questioned this assumption, maintaining that cod liver oil is more efficacious than viosterol in preventing rickets and restoring normal calcification. That this latter view is not reasonable is evident from the fact that the antirachitic potency of viosterol is determined by comparison with the antirachitic potency of the average cod liver oil. Except in leg weakness in chickens (2), the antirachitic potency of the two substances appears to be identical under the same experimental conditions. When these conditions are varied, comparable results are not to be expected. Thus, the comparison of the activity of the two substances in human beings under different conditions of diet and environment is not entirely justifiable, unless the observations are based on a very large number of cases.

That viosterol and cod liver oil are equally potent in preventing and curing rickets in rats on high calcium-low phosphorus diets is well established and this fact is utilized in determining the therapeutic activity of viosterol. That both substances are equally effective in protecting the body against skeletal abnormalities resulting from feeding low calcium-optimal phosphorus diets has not been determined.

It was previously shown by Shipley, Park, McCollum and Simmonds (3), that rats reared on diets deficient in calcium, but otherwise adequate, failed to grow normally and that their skeletons showed osteo-

[1] Aided by a grant from Mead Johnson and Company, Evansville, Indiana.

porosis. When cod liver oil was added to such diets growth was markedly improved and the skeletal abnormalities were prevented. Butter fat, however, failed to correct the lime salt deficiency, even when fed fifteen times the amount necessary to protect animals against xerophthalmia. The experiments suggested that cod liver oil, in contrast to butter fat, is capable of protecting the body against calcium deficiences. The beneficial effect was attributed to the abundance of vitamin D in the cod liver oil. The following experiments, suggested by Professor E. V. McCollum, were aimed to determine the compara-

TABLE 1

Composition of diets used in experiments 1, 2 and 4

BASAL DIET 1A		DIET 1A	DIET 1B	DIET 1C	DIET 1D
Wheat..... 78.5	Series 1, no vitamin D addition	Basal diet, no calcium addition	Basal diet +0.2 gm. per cent $CaCO_3$	Basal diet +0.4 gm. per cent $CaCO_3$	Basal diet +0.6 gm. per cent $CaCO_3$
Casein (purified)..... 10.0	Series 2, 2 per cent C.L.O.*	2A	2B	2C	2D
Salts (Ca-free)..... 2.5	Series 3, 4 per cent C.L.O.	3A	3B	3C	3D
Butter (washed)..... 5.0	Series 4, 2 per cent viosterol 1D†	4A	4B	4C	4D
Olive oil..... 4.0	Series 5, 4 per cent viosterol 1D	5A	5B	5C	5D
100.0	Ca, gm. per cent =	0.050	0.130	0.210	0.290

* C.L.O. = Cod liver oil.

† Viosterol was diluted to equal cod liver oil in vitamin D potency.

tive effectiveness of viosterol and of cod liver oil in maintaining the growth and health of rats when their dietary calcium was inadequate.

EXPERIMENTAL

The plan of the experiment was as follows: A basal diet which was lacking in vitamin D and low in calcium but adequate in all other respects was chosen. It was composed of wheat, 78.5 per cent, casein (purified) 10 per cent, a calcium-free salt mixture 2.5 per cent, butter 5 per cent and olive oil 4 per cent. The calcium content of the diet was

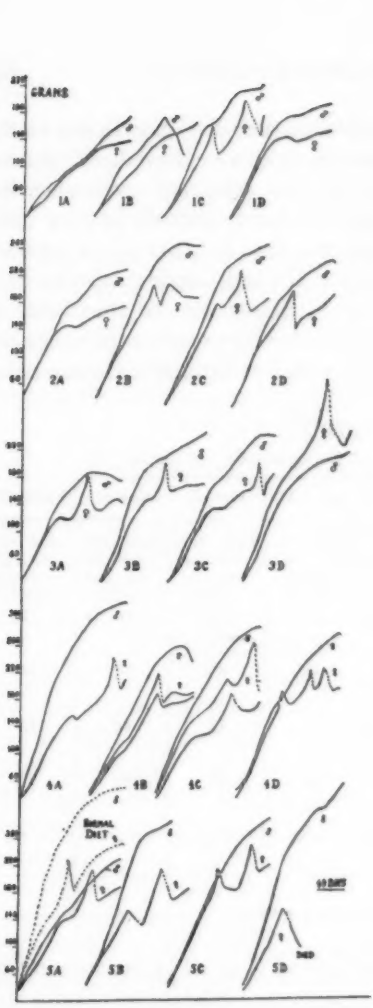

FIG. 1

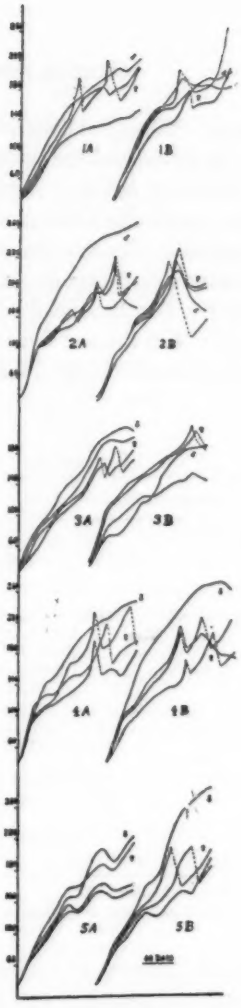

FIG. 2

FIG. 1. GROWTH CURVES OF ANIMALS IN EXPERIMENT 1

Series 1, no vitamin D. Series 2, 2 per cent C.L.O. Series 3, 4 per cent C.L.O. Series 4, 2 per cent viosterol 1D. Series 5, 4 per cent viosterol 1D.

Diet A, no $CaCO_3$ added. Diet B, 0.2 per cent $CaCO_3$ added. Diet C, 0.4 per cent $CaCO_3$ added. Diet D, 0.6 per cent $CaCO_3$ added.

Dotted line denotes birth of litter.

FIG. 2. GROWTH CURVES OF ANIMALS IN EXPERIMENT 2

Designation same as in figure 1

approximately 0.050 gm. per cent (Table 1). This basal diet, which, as seen, was extremely deficient in calcium, was designated as 1A. From it, by means of the addition of increments of 0.2 per cent $CaCO_3$, three other diets, designated 1B, 1C and 1D were constructed. Diet 1B was basal diet 1A + 0.2 per cent $CaCO_3$ (Ca = 0.130 per cent); diet 1C was basal diet 1A + 0.4 per cent $CaCO_3$ (Ca = 0.210 per cent); and diet 1D was basal diet 1A + 0.6 per cent $CaCO_3$ (Ca = 0.290 per cent). The four diets 1A, 1B, 1C and 1D constituted diet series I. Through the addition to each of these four diets of 2 per cent cod liver oil (series 2), 4 per cent cod liver oil (series 3) 2 per

TABLE 2

Composition of diets used in experiments 3 and 5

Basal diet 1K:		
Wheat	40.0	Diet 2K, basal diet +2 per cent C.L.O.
Wheat gluten	5.0	Diet 3K, basal diet +4 per cent C.L.O.
Salts (Ca-free)	2.5	Diet 4K, basal diet +2 per cent viosterol 1D*
Butter (washed)	5.0	Diet 5K, basal diet +4 per cent viosterol 1D
Olive oil	4.0	
Starch	35.5	
	100.0	

Ca = 0.012 gm. per cent.

* Viosterol was diluted to equal cod liver oil in vitamin D potency.

cent viosterol (series 4) and 4 per cent viosterol (series 5), four other series of four diets each were established. In this way 20 diets were obtained presenting a range of deficiency in calcium and fortified with comparable amounts of cod liver oil and viosterol. In experiment 1, a male and female rat 25 days old and weighing between 40 and 50 grams were placed on each of the 20 diets and kept on the diet for 150 days. In experiment 2 three females and 1 male of the same ages and weights as those just mentioned were used and only diets A and B, containing no added calcium and 0.2 gm. per cent $CaCO_3$, were fed for 150 days. Viosterol and cod liver oil were fed at the same levels as in experiment 1 (Fig. 2).

On the conclusion of experiments 1 and 2 it seemed advisable to make the conditions more drastic by increasing the calcium deficiency. Accordingly a new basal diet, 1 K, was devised which contained only 0.012 gm. per cent of calcium. The lowering of the calcium content of the diet was accomplished by substituting 5.0 gm. of wheat gluten for 38.5 gm. of wheat flour in basal diet 1A and making up the difference

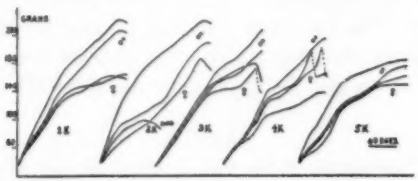

FIG. 3. GROWTH CURVES OF ANIMALS IN EXPERIMENT 3

1K, no vitamin D added; 2K, 2 per cent C.L.O.; 3K, 4 per cent C.L.O.; 4K, 2 per cent viosterol 1D; 5K, 4 per cent viosterol 1D.

FIG. 4. GROWTH OF ANIMALS IN EXPERIMENT 4

Designations same as in Figure 1

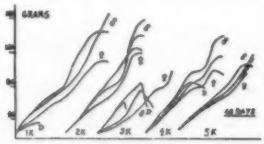

FIG. 5. GROWTH CURVES OF ANIMALS IN EXPERIMENT 5

Designations same as in figure 3. D = Died

with starch. The protein content of the diet was little altered, since gluten contains 8 times as much protein as wheat flour. In experiment 3, groups of two males and two females 25 days old and weighing 40 to 50 gm. were kept for 150 days on basal diet 1K alone and supplemented with 2 per cent and 4 per cent cod liver oil and viosterol, respectively (Table 2 and Fig. 3). In experiments 4 and 5, the calcium defi-

ciency was made even more drastic by placing the animals on the calcium deficient diets when they were about 16 to 18 days old and weighing only 25 gm. Thus the factor of calcium storage was minimized and any beneficial effect of viosterol or cod liver oil would be more apparent. Experiment 4 is a repetition of part of experiment 2 and experiment 5 is similar to experiment 3.

In all the experiments here reported the animals were housed in wire cages with raised meshed wire flooring in a ventilated room which was dark, though not completely free from daylight, and at times illuminated by an ordinary electric light. The animals were weighed weekly. The food and water containers were changed daily. Growth curves were plotted and, whenever possible, at the time the animals were killed, blood was obtained for analysis for calcium and inorganic phosphorus.

RESULTS

1. *Effect on growth.* It is seen from figures 1 and 2 that animals reared on diets deficient in calcium and vitamin D, but otherwise adequate, failed to grow normally. Growth was improved with increasing amounts of calcium in the diets, but normal growth was never attained since the diet containing the largest amount of calcium (0.290 gm. per cent) was below the optimal requirement for normal growth and reproduction. A decided improvement in growth, reproduction and well-being was noted in the groups receiving vitamin D. There was no appreciable difference between the cod liver oil and viosterol groups; and, if judgment were permissible on small numbers of animals, the viosterol groups would be adjudged the more vigorous, in spite of the lesser content of vitamin A in their diets as compared to the diets of the cod liver groups.

In experiment 3 (Fig. 3) the calcium content of the diet was only 0.012 gm. per cent and growth was much below normal and it was not improved by the addition of vitamin D.

In experiments 4 and 5 the calcium deficiency was made very drastic by placing the animals on the prescribed diets when they were about 16 to 18 days old and weighing only about 25 gm. and hence their growth was exceedingly poor. In experiment 4 the addition of 0.2 gm. per cent $CaCO_3$ improved the growth of the animals to some extent, but

in both experiment 4 and 5 cod liver oil or viosterol were of little avail in overcoming the extreme calcium deficiency.

2. *Effect on reproduction.* Animals in experiments 4 and 5 never reproduced. This was probably due to their extremely low reserves and low intake of calcium. In experiment 3 there was one litter born to a female on a diet containing 4 per cent cod liver oil (3K) and a litter each to two females on the same diet with 2 per cent viosterol (4K). In the first two experiments, reproduction improved with the increase of calcium and vitamin D in the diets. Animals in experiment 3 reproduced several times, especially on diets containing vitamin D. The offspring of these animals were, however, smaller at weaning than those born of parents reared on our stock diet. Their average weight at 20 days was 20 to 25 gm. as compared to 30 to 35 gm., the average weight of normal offspring at this age and the same number in liters. Many "killers" (mothers killing their offspring) were present among all groups, especially among animals subsisting on an extremely low calcium diet. Some of the suckling females lost considerable weight during the lactating period; and one died as a result of calcium loss through lactation (Experiment 2, 5D).

In general, there was little difference in reproduction between the cod liver oil and viosterol groups.

3. *Effect on serum calcium and inorganic phosphorus.* The concentration of serum calcium in the normal animal is fairly constant even under varying conditions of calcium intake. Its concentration in the blood can only be altered appreciably by lowering the calcium and markedly increasing the phosphorus in the diet; by derangements of calcium and phosphorus metabolism in rickets and tetany; by changes in the serum protein concentration in certain types of nephropathies; by parathyroidectomy; by idiopathic hyper and hypo-function of the parathyroid glands and by the injection of parathormone. The constancy of the serum and calcium in normal adult human beings on a low calcium intake over short periods has been previously observed (4, 5). That the serum calcium does not fall to tetanic levels in *growing* rats reared on low calcium-optimal phosphorus diets over long periods of time may be seen from Table 3. In some cases, the concentrations of serum calcium were normal and in others at low normal levels. In experiment 5 the calcium reserves of the animals were very

low and their rations contained only 0.012 gm. per cent of calcium and their serum calcium concentrations were only somewhat lower than normal except in one instance in the group not receiving vitamin D.

In contrast, the serum concentration in hypoparathyroidism fluctuates with the calcium intake (6), and frequently normal serum calcium levels may be observed, although the clinical evidence may point to a deficiency of parathyroid function. The normal serum cal-

TABLE 3

Calcium and inorganic phosphorus concentrations of blood serum of the different groups

Values expressed in mgm. per cent

	1A	2A	3A	4A	5A	
Ca	11.8	10.0	9.1	10.0	10.5	9.8
P	6.9	6.5	5.9	4.8	5.9	4.8
	1B	2B	3B	4B	5B	
Ca	10.9	11.1	10.5	11.5	10.4	12.1
P	4.9	6.2	4.5	4.5	7.2	5.7
	1C		3C		5C	
Ca	10.9		9.5		10.9	
P	4.7		5.4		6.2	

	EXPERIMENT 3					EXPERIMENT 5				
	1K	2K	3K	4K	5K	1K	2K	3K	4K	5K
Ca	11.2	10.8	10.2	12.5	13.1	8.9 6.7	8.2	9.5	9.5	9.0
P	7.8	9.8	8.0	5.4	6.7	6.6 8.1	5.9	8.0	6.0	5.3

cium level may be due to an adequate calcium intake; and if the calcium of the diet is reduced appreciably for a few days and the blood for calcium determination is drawn after twelve hours fasting, the concentration of serum calcium may be reduced. This variation in serum calcium occurs not only in parathyroidectomized rats but it was also observed in patients studied on the medical service in whom hypoparathyroidism was suspected. Their blood serum calcium fluctuated

between normal and tetanic levels concomitant with the calcium and phosphorus intake (7).

The serum inorganic phosphorus varied too widely to warrant any definite deductions. In general, the concentrations ranged between normal and quite high levels, but never lower than normal concentrations. There was no definite correlation between the serum calcium concentrations and the serum inorganic phosphorus levels. The concentration of these elements in the serum in the animals in the viosterol groups was slightly higher than the cod liver oil groups, especially with respect to calcium.

4. *Effect on skeleton.* The effects of low calcium diets on the skeletons of growing rats have been described by many observers, notably McCollum, Simmonds, Shipley and Park (8). The lesions in our animals resembled in many respects those found by them. The long bones were osteoporotic. The heads were soft and easily compressible. The thoraces were soft, flattened out and cut very easily. The ribs showed evidence of many fractures. The degree of skeletal abnormalities varied with the calcium and vitamin D intake and calcium storage. It was most pronounced in experiments 4 and 5 and in the animals receiving no added calcium or vitamin D in their diets. The long bones of these animals were slender, thinned out, and broke very easily on the slightest manipulation. They were soft and cut with ease. The metaphyses were narrow and the trabeculae few in number. Improvement in skeletal development was noted in the animals receiving calcium and vitamin D, but it never approached normal ossification. There was no demonstrable difference in skeletal structure in the various groups receiving viosterol or cod liver oil.

5. *Effect on teeth.* The effect of diet on tooth structure and dental caries has recently attracted much attention. The most extensive studies are those of Klein (9), M. Mellanby (10), and McCollum and Grieves (11). Klein studied dentition of growing pigs on low calcium diets and observed maldevelopment of the permanent as well as the deciduous teeth. He was unable, however, to find dental caries in the molars of his experimental animals except in a few instances. M. Mellanby studied the effect of various diets, usually of the low calcium variety, on the dentition of dogs. She suggests that faulty dentition occasioned by diets poor in calcium might predispose to the formation

of dental caries. Her experimental data, however, in no way warrant such suggestions since no caries were ever observed in her animals; and E. Mellanby (12) admits, in a recent publication, the absence of caries in the animals studied by M. Mellanby. McCollum and Grieves' analysis of dental defects in rats on various diets show that the incidence of dental caries is less frequent on calcium poor diets than on rations deficient in other respects.

The teeth in all our series of animals were poor in structure; the degree of abnormality varying with the calcium and vitamin D intake, but they showed no gross caries. The histologic tooth structure of the animals on the various diets is being studied in collaboration with Dr. Henry Klein of the Department of Chemical Hygiene of the School of Hygiene and the results will be reported in detail elsewhere.

6. *Effect on hair.* In the groups receiving no added calcium in their rations, hair growth was exceedingly poor. Some of the animals lost their long hair and were covered with a coat of thin, short, fuzzy hair; others lost their hair in areas, most frequently around the abdomen and extremities. The hair loss was more marked in animals on low calcium diets containing no vitamin D and in groups failing to grow. It was less noticeable in the groups receiving added calcium in their diets and nearly absent in those receiving added calcium and vitamin D.

7. *Pica and coprophagia.* There was tendency for pica and coprophagia in most of the animals. Care had to be exercised on opening cages, while changing food and water jars, not to allow the rats to leave the cages. As soon as one left a cage he would attempt to eat feces from under the cages. Osteophagia was evident on several occasions when young rats were introduced in the same cages with the older rats kept on low calcium diets. Within a minute screams were heard and the older rats were found killing the young rats by biting them about the nape of the neck and sucking their blood. Considerable difficulty was encountered in attempting to rescue the struggling victims, as the killers refused to part with their prey.

Such perverted metabolism is not infrequently found among cattle of certain sections of Africa and Minnesota (13, 14). Cows may become carniverous if they are forced to subsist on vegetations low in lime salts and they will eat bones of cattle or other mammals for their lime salts content.

Similarly, wire eating has been observed in lactating cows fed on diet deficient in iron and frequently wire eating is the cause of perforation of the stomachs of cattle (15).

DISCUSSION

The deleterious effects of calcium deficient diets on growing rats have been discussed at length by McCollum and his co-workers. They have also demonstrated the efficacy of cod liver oil as against butter fat in protecting the animals, in part, against the calcium deprivation. Cod liver oil administration promoted retention of the small amounts of calcium ingested and resulted in fairly good growth, skeletal development, reproduction and lactation. The basal diets employed in some of the present experiments were far more deficient in calcium than McCollum's rations and hence neither cod liver oil nor viosterol restored normal growth and reproduction in these animals unless some calcium was added to their rations. The deficiency was quite marked in experiments 4 and 5 when the animals' stores of calcium were minimized by placing them on the experimental rations at an early age. With increasing amounts of calcium there was improvement in the growth and well-being of the animals; and they were further benefited by the addition of vitamin D to their rations. There was, however, no detectable difference between the biologic response of the animals to cod liver oil and viosterol.

It is quite remarkable how the growing organism can withstand a calcium deficiency for such long periods of time. In experiments 3 and 5, although the calcium of the diet was only 0.012 gm. per cent and the bones were osteoporotic and contained many fractures, yet many of the animals made a fair gain in weight and survived many months. In similar experiments with optimal calcium—low phosphorus diets (16) it was found that growth was hardly possible even if the concentration of phosphorus in the diets was 8 to 10 times that of the concentration of calcium in the calcium-poor diet. This may be attributed to the relative difference in the number of physiologic functions of the two substances. While most of the calcium in the body is concerned with the ossification and with the maintenance of a normal response to nervous stimuli, the known functions of phosphorus are more numerous. Not only is it equally essential as calcium in bone

formation, but it is a most important element in the building, function and intermediate metabolism of muscle, gland and nervous tissue. For this reason a deficiency in the phosphorus intake is reflected not only in the osseous structure but also in growth and maintenance.

The experiments here reported seem to indicate that cod liver oil and viosterol of comparable vitamin D potency, are equally effective in preventing calcium loss from the body under similar conditions of experiment.

SUMMARY AND CONCLUSIONS

1. There is no demonstrable difference in the physiological effects of cod liver oil and viosterol in sparing body calcium in rats fed diets adequate in all respects except for a paucity in calcium.

2. Growth and reproduction is improved with increase in calcium and vitamin D of the diets.

3. Younger rats are more susceptible to a calcium deficiency in the diet than older rats.

4. The serum calcium levels of rats reared on low calcium diets for long periods is within the normal limits. It is slightly reduced in the serum of animals placed on the diets at a very early age. It is somewhat higher in the groups receiving viosterol in their rations.

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